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Genetic Analysis and the Ethics of Secondary Findings

Jacob Zieba
DePauw University

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Sponsor: Daniel Gurnon

Committee: Daniel Gurnon, Rebecca Upton, Erik Wielenberg, Lynn Bedard

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Introduction

The power and influence of genetic testing is demonstrated in a case that involved a three-year-old male with a pregnant mother who came in to a genetic counseling clinic with symptoms of Mucopolysaccharidosis type II (MPS type II); also known as Hunter's syndrome. This disease is caused by a deficiency in the lysosomal enzyme iduronate 2-sulfatase which leads to a buildup of glycosaminoglucans in cells all over the body and almost exclusively affects males. The effect of this is abnormal development during key formative years and begins to show around ages 2-4. Patient's exhibit troubled breathing, swelling, skeletal malformations and neurological degeneration. Most patients with this disease only live until around age 20 with treatment.[1] MPS is an X chromosome linked disease meaning the mother carries the mutation but, in this case, does not experience symptoms herself because of her second X chromosome. After diagnosis of the child, the mother was tested and found to be a carrier of the disease meaning she had a 50% chance of passing on the disease to her next male child. The mother was in fact thirty-four weeks pregnant at the time of diagnosis with another male child. The fetus was also tested and found to be affected by the disease. After deliberation, the parents chose to carry out the pregnancy but gave up the

[1]Wraith, J. Edmond, Maurizio Scarpa, Michael Beck, Olaf A. Bodamer, Linda De Meirleir, Nathalie Guffon, Allan Meldgaard Lund, Gunilla Malm, Ans T. Van der Ploeg, and Jiri Zeman. 2008. "Mucopolysaccharidosis Type II (Hunter Syndrome): A Clinical Review and Recommendations for Treatment in the Era of Enzyme Replacement Therapy." *European Journal of Pediatrics* 167 (3): 267–77. doi:10.1007/s00431-007-0635-4.

child for adoption because of the disease and treatment began on both children at the same clinic. Unexpected findings from genetic testing has a huge impact in the lives of patients. This is the reality that many patients who undergo genome sequencing face and it is a problem that researchers of genetics and clinicians alike must address.

Stories like this show the importance and power of genomic data in the lives of everyday individuals. The mother was made aware of her unborn child's condition because of incidental findings that affected her and the child. Next-Generation Sequencing is the latest and most widely used series of techniques by researchers and clinicians alike to study heritable diseases like MPS. Genome sequencing allows researchers to narrow down the problem in the genome which translates into various things down the line like individual proteins or organ development. Easy genomic testing did not come about until 1977 with the advent of Sanger sequencing. First generation technologies like this allowed for researchers to slowly test aspects of a genome. It was not until 2005 with the Genome Analyzer that allowed for sequencing runs to go from 84 kilobase per run to 1 gigabase per run that easy genome sequencing was possible. This was the start of the "Next" generation of sequencing. The first human genome to be sequenced and published was in 2001 and took 15 years and 3 billion dollars to complete. By 2014, the cost for an individual's genome to be sequenced dropped to 1000$ and can be done in a single day.[2]

[2] "An Introduction to Next-Generation Sequencing Technology." 2016. http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf.

Genetic information is becoming more widely used and it has many applications that go beyond diagnosing a single disease in an individual. Because we have the ability to analyze the entire set of an individual's genetic information even though we are looking for something specific, there is the chance of finding other problems. These other problems that we find have the ability to severely affect the patient whose genome we are sequencing. For example, if we found out a patient is likely to experience a deadly heart condition or cancer in the future while searching for an unknown metabolic disorder. These possible findings are called secondary findings and are an ever-increasing issue in in the field of genetics. Secondary findings are findings that concern a patient's health or are reproductively significant that are not under the scope of the research being conducted.[3] Issues surround the ability for researchers to fully inform patients as well as addressing the uncertainty of developing a disease; even with mutated genes. A large part of the discussion is about the diseases and genes that should be included or not when reporting back to patients with the final analysis. There is also the issue of calculating the severity of the diseases that should be included within reports. These are some of the questions that must be addressed when discussing secondary findings.

I will argue that the best approach for researchers and research institutions when dealing with secondary findings is to develop an ease of information so that patients are fully

[3] Wolf, Susan M, Brittney N Crock, Brian Van Ness, Frances Lawrenz, Jeffrey P Kahn, Laura M Beskow, Mildred K Cho, et al. "Managing Incidental Findings and Research Results in Genomic Research Involving Biobanks and Archived Data Sets." *Genetics in Medicine : Official Journal of the American College of Medical Genetics* 14, no. 4 (April 2012): 361–84. doi:10.1038/gim.2012.23.

informed going into genetic analysis. The autonomy of the patient is incredibly important when dealing with requesting secondary findings and in almost all cases, the decision of the patient is final. Within this thesis, I will be recommending where and how to present secondary findings to patients and to the Rare Genomics Institute (RG) that works with rare disease patients from all across the country. I will be recommending genes from the American College for Medical Genetics secondary findings list as well as my own research to be included within these secondary findings reports. The goal is to provide a useful and comprehensive recommendation for RG to start reporting secondary findings to patients so that they will be even more helpful to patients who contact this non-profit research institution.

Patients often seek help in a medical clinic, where a clinician communicates with genetics counseling institutions like RG or guides the patient to contact these institutions themselves. Patients are then recommended a genetics counselor that helps the patient get sequenced. After sequencing and initial analysis, the genomic data is sent to these institutions for further analysis. Institutions like RG pass along the information to registered volunteers who comb through the data of a patient in order to report back to the institution which then contacts the patient or the patient's clinician. This relationship complicates ethical analysis because volunteers are not medical professionals but are effectively held to a high standard. Volunteers are given the patient's exome as well as the immediate family's exomes and are the primary data miners. They give their findings to the institute with the

recommendation to report or not. In a non-reported case, the results are generally inconclusive as one or two variants were not deemed to be primarily causative in the patient. Volunteers use databases like Omicia Opal and OMIM (Online Mendelian Inheritance in Man database) to analyze the results and reactions of mutations. Omicia comes with analysis from other databases as well as its own score on the whether a mutation is likely to be deleterious or not named the Omicia score. This score lies between 0.00 and 1 where the higher integers indicate likelihood of deleteriousness. Each gene has an Omicia score and the database allows for sorting via Omicia score and other factors that indicate deleteriousness in a gene.

Through these indicators, geneticists and volunteers determine the chances of a gene being causative and report this kind of information. The responsibility of relaying the findings of the researchers to the patient is the patient advocate or clinician and informed consent is usually obtained this way as well. Clinicians are often grouped with genetics researchers in discussions on secondary findings, however they maintain a distinctly separate role. Clinicians act as a middleman that help patients be informed and sometimes keep contact with the researching institution. The primary party responsible for the ethical foundations and work are the researchers who take the genomic information to analyze. They are the ones responsible for informing the patients and protecting the information gathered.

There is a broad discussion over the significance of secondary findings and whether researchers are required to report these findings. The general consensus amongst clinicians, researchers and patients is that these findings should be made available to the patient but many problems exist between the actors in this ethical quandary.[4] From the patient's perspective, there is the question of the rights of autonomy that exist between the information that researchers can provide and future problems that could be mitigated from this knowledge. Informed consent also creates a situation between patients and researchers where it is the researcher's responsibility to fully inform the patient to the best of their ability, with or without the help of a consulting clinician. There is also the issue of what researchers would be required to report if they are required to at all. Whole exome sequencing is limited in its ability to definitely pin a cause to certain diseases as it is only about 1-2% of the total genome. Recent research has also indicated that the exome is not the only part of the genome responsible for problems, demonstrating that whole exome sequencing is limited in some significant ways. The criteria for causative mutations are still being researched so choosing which genes to report is a tremendous scientific task. Some institutions like the Rare Genomics Institute, do not currently engage in secondary findings which effectively rob patients of valuable information that could contemporarily be presented to them. Through informed consent, respect for autonomy and critical gene analysis these institutions can effectively provide a means for patients to maximize the

[4] Klitzman, R, P S Appelbaum, A Fyer, J Martinez, B Buquez, J Wynn, C R Waldman, J Phelan, E Parens, and W K Chung. 2013. "Researchers' Views on Return of Incidental Genomic Research Results: Qualitative and Quantitative Findings." *Genet Med* 15 (11): 888–95. doi:10.1038/gim.2013.87.

effectiveness and beneficence of genome sequencing. As a primer for the rest of the thesis, this next chapter is a quick overview of genetics that the reader will need to understand later chapters.

An Introduction to Genetic Analysis

Hereditary diseases are a result of malfunctions in the complicated machine that is a human being. DNA (Deoxyribonucleic Acids) is the foundation of this machine and genetics as a whole. Hidden within DNA is the code that tells the cogs within the machine how to develop and work; it is the blueprint of the human organism. We knew that DNA was the blueprint for organisms but the structure of DNA was not discovered until 1953 when James Watson and Francis Crick published a paper in *Nature* on the subject with heavy influence from Rosalind Franklin.[5] The blueprints turned out to be only four nucleotides, equivalent to four letters. The four letter code that makes up DNA was not broken until 1960s when scientists like Marshall Nirenburg and Johann H. Matteai discovered how information was stored in these strands.[6]

[5] "Franklin, Watson & Crick." 2010. http://virtuallaboratory.colorado.edu/Biofundamentals/labs/WhatisScience/section_08.html.
[6] "Timeline: History of Genomics." 2016. *Wellcome Genome Campus*. http://www.yourgenome.org/facts/timeline-history-of-genomics.

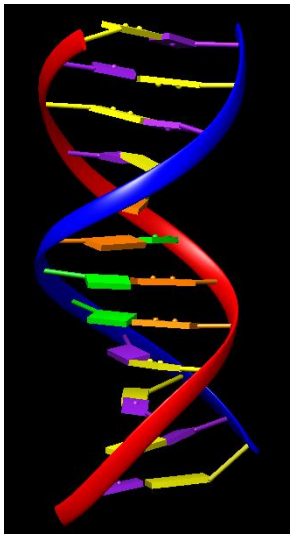

Figure 1: Chimera representation of B-Deoxyribonucleic Acid (DNA). The red and blue strands are the backbone of the molecule. The central rectangles represent the four sugars equivalent to the letters A, T, C, G.

The blueprints are read in triplets, like CAT or TAG, which code for different amino acids. Amino acids are the next building block which come together to make up proteins. One can think of proteins as the tiny cogs in the human machine. There is an incredible number of these cogs within the human machine and the DNA blueprints that buildup everything else are essentially organized into sections called genes.[7] Genes are long sequences of those triplets which can be tens of thousands of letters long. Genes are divided into two different sections considered coding and non-coding parts of the gene; like usable and non-usable parts. Exons are the parts of the gene that remains after certain parts of the transcripted gene are cut out. Exons are the protein encoding parts of the DNA and are essential in both life and medical research and the total combination of exons is called the exome. This contrasts with the genome, in that, the exome is the protein coding parts of the DNA and the genome is the entire set of DNA; coding and non-coding parts.

[7] "An Overview of the Human Genome Project." 2015. *National Human Genome Research Institute*. https://www.genome.gov/12011238.

The genome is packed into every cell and the genome itself is divided into 23 pairs of compact DNA called chromosomes. If we are thinking about machines, then chromosomes can be compared to essential car parts where one chromosome would code for the different parts of the engine in a car and another would code for the parts that make up the tires. Chromosomes are more complicated than this in that one chromosome could have parts of the engine, tires and transmission within it. One missing pair of chromosomes and the car cannot be completed. All cells have two copies of each chromosome inheriting one from each parent. The parents produce haploid gametes which are cells that only have one copy of each chromosome. Each copy of the chromosome inherited from the parents differs slightly. What people know as traits are the outward expression of the genes on these chromosomes like the color and form of the car. Traits are known to be recessive or dominant where recessive traits are those that result of two abnormal alleles being inherited. Dominant traits are those where only one allele is necessary to exhibit the trait so for example, brown eyes are more common in the general population because the alleles that encode for brown eyes are dominant. An allele is a variant form of a gene which is brought about by mutations. Some mutations are benign and do not change the function of the protein coded by the gene whereas others are problematic and change or eliminate the function of the protein coded by the gene. If one copy of a chromosome within an individual has a deleterious mutation, there is usually another copy with the non-mutated gene that corrects for the mistake. If someone inherits two of the same allele with the same mutation in both of the inherited chromosomes, then it is known as homozygous. If someone inherits two different,

deleterious mutations then it is known as compound heterozygous. These mutations sometimes manifest themselves as hereditary disorders and are the motivation for medical genomic sequencing which brings about the topic issue of secondary findings.

Patterns of inheritance are how genes with mutations in them get passed down onto the next generation. Autosomal dominant means only one mutated form of the gene needs to be present to cause the disease whereas two mutated genes need to be present for autosomal recessive inheritance. X-linked inheritance is affected by dominant and recessive inheritance but is also the only chromosome that is sex dependent which affects who may experience the disease. If a male inherits a mutated gene on the X chromosome, they do not have another copy like females do to correct for the non-functional protein. Somatic mutations are mutations that occur after conception and multifactorial inheritance means that many factors play into whether the disease is present or not. Multifactorial inheritance can be a combination of genetic and environment factors so the disease may not be entirely genetic.

Modern methods of genomic sequencing highlight entire exomes or genomes which are invaluable to researchers, clinicians and patients.[8][9] New Generation Sequencing (NGS) is sweeping the market of genomic sequencing leading to lower costs and more availability for

[8] Appelbaum, Paul S., Erik Parens, Cameron R. Waldman, Robert Klitzman, Abby Fyer, Josue Martinez, W. Nicholson Price, and Wendy K. Chung. "Models of Consent to Return of Incidental Findings in Genomic Research." *Hastings Center Report* 44, no. 4 (2014): 22–32. doi:10.1002/hast.328.

[9] Olson, S, S H Beachy, C F Giammaria, and A C Berger. *Integrating Large-Scale Genomic Information Into Clinical Practice: Workshop Summary*, 2012.

researchers and medical professionals.[10] The ability to sequence an entire genome the size of humans is only a recent development. Frederick Sanger sequenced the first genome of a virus in 1977 and the first disease associated gene was found in 1983. The Human Genome Project was launched in 1990 with the goal of sequencing and mapping all the genes within humans and this goal was completed in 2003.[11] This lead to comparable collections of human genomes like the 1000 Genome Project which researchers use as templates for genetic study.[12] The culmination of sequencing technologies has allowed clinicians and researchers to analyze a patient's genome within just a few months. Analyzing a subject's entire genome allows genetics professionals to establish the precise causes or influences of certain diseases. Similarly, whole exome sequencing provides another tool in establishing these causes and is an easier way to determine causative DNA mutations. There is a vast quantity of data that genome sequencing creates which researchers and clinicians must sort through which creates the issue of secondary findings. This takes many forms from pharmacogenetics to genetic counseling where many fields conduct research using whole genome and exome sequencing. The problem is two-fold in how we should approach secondary findings and what should we report to patients. The object of this thesis is to explore and recommend ethical issues surrounding secondary findings for genetic researchers at the Rare Genomics Institute and to

[10] Morozova, Olena, and Marco A. Marra. "Applications of Next-Generation Sequencing Technologies in Functional Genomics." *Genomics* 92, no. 5 (2008): 255–64. doi:10.1016/j.ygeno.2008.07.001.

[11] "All About The Human Genome Project (HGP)." *National Human Genome Research Institute*, 2015. https://www.genome.gov/10001772.

[12] "Timeline: History of Genomics."

others that provide services to patients with rare genetic diseases; as well as suggest which genes should be considered in patient reports.

Next Generation Sequencing is expanding the field of genetics at an incredible speed but at the current time there are still many limitations that we as researchers are bound by and affect reporting secondary findings. NGS sequencing is still a relatively new technology so there is a limit on the knowledge that researchers have on the genome and genes themselves. Not everything is known about some genes like how they function and interact with other genes. Oftentimes databases will mark mutations in a gene as a "variant of unknown significance" which is just a mutation whose ramifications are yet unknown to researchers. Research and scientists are not always correct in their analysis and problems can develop in reporting secondary findings from simple mistakes. Unknowingly reporting that a certain mutation within a gene is benign when in reality it has deleterious effects could be a severe mistake to report to patients. It is not unknown for the significance of a mutation to change from benign to pathogenic or vice versa. Databases can compound this problem like Clinvar. Clinvar is a free use database that reports relationships among human variation and phenotypes with supporting evidence. Clinvar works by integrating multiple other databases and allows for researchers to report their data. The problems that arise from this aggregation of sources is that other databases may misreport the pathogenicity of a mutation which then shows up in Clinvar and pathogenicity reports. Because Clinvar is free it may discourage researchers from further studying whether a specific mutation is pathogenic or not which

could spread misinformation. Databases like Omicia Opal also have problems that stem from its operations. Omicia is a comprehensive analysis tool for exomes and whole genomes which show the entire exome and can be compared to others. Omicia uses databases like Clinvar and COSMIC for its own evaluation of the deleteriousness of gene called the Omicia Score. Institutes like RG use Omicia Opal for much of their analysis and if other aggregate databases are wrong about a gene then the misinformation may be given to patients as diagnoses. A lot of questions surrounding secondary findings stem from these uncertainties but the likelihood of these database problems is very low and the databases are a great and expediting tool for genetic analysis that benefits the process of genetic analysis.

Informed Consent

Obtaining permission from the patient is the first incredibly important step when it comes to analyzing their genomes. Genetic information can be stored indefinitely and can be reevaluated after new genetic discoveries. Storing genetic data is invaluable for researchers, clinicians and patients alike and there are many ways organizations are storing this kind of data. The Personal Genome Project is a research project devoted to sharing genomic data for these reasons for the benefit of patients and research.[13] Patients are also likely to want discloser of secondary findings or data that is relevant to family members. The genome of one individual can help warn of risks for both that patient and other members within their

[13] Lunshof JE, and Ball MP. "Our Genomes Today: Time to Be Clear," 2013. doi:10.1186/gm52.

immediate family. There are many possible approaches for patients after they sequence their genome however it is often an uphill battle in informing patients to an acceptable degree where they understand what is being asked of and done with their genetic data.

Informed consent is the autonomous and informed authorization by the patient for participation in research. Clinicians generally have the closest contact with the patients and their families which, at least on the surface, puts the responsibility of informed consent on the shoulders of the practitioner clinician. Informed consent is the condition of a patient or subject having substantial understanding of the process and autonomously authorizes professionals to work with them.[14] As an ethical standard for medicine, informed consent is necessary when analyzing an individual's genetic information. Basic moral concepts that surround the issue of informed consent being; nonmaleficience, beneficience, justice, and respect for autonomy. The general approach to many medical decisions is being as ethical as possible when dealing with patients. This approach has been modified from the Hippocratic Oath and is the general principle of doing no harm[15] which is the core of nonmaleficience. The express goal of clinical work and medical research is to help patients and stop whatever harm has befallen them. Similarly, the principle of beneficence is the notion that there is an obligation to act for benefit of others, and in this case patients, and justice is the right to this treatment.[16] The principle of justice is all about the access to healthcare and information.

[14] Beauchamp, Tom L., and James F. Childress. *Principles of Biomedical Ethics: Fifth Edition*. Oxford University Press, 2001. 78.
[15] Beauchamp and Childress, *Principles*, 114
[16] Beauchamp and Childress, *Principles*, 166, 226

With this principle, it is important to provide as much information to the patient as possible, within reason, which is why secondary findings should always be offered. In ignoring secondary findings, institutions are not doing the patient any justice by robbing them of valuable information. These are ethics principles that are going to be assumed to be standard regarding secondary findings.

The importance of informed consent is evident in the problems that have arisen in the past from unethical medical and research practices. In 1958, a pancreatic enlargement patient that entered medical care and was recommended by the practitioner to have a cystoscopic exam. After this exam, the practitioner recommended a transurethral prostatic resection the next day which makes the patient sterile. The patient was not informed of the resulting sterilization before the procedure and sued the hospital in the famous Bang vs. Charles T. Miller Hospital case.[1718] This case demonstrates the consequences of a severe breach in informed consent. In less extreme cases, there is still a difference between adequately informed and fully informed. This poses a unique problem where a patient should clearly be fully informed of the medical professional's intentions but there may be situations where practitioners and researchers can only adequately inform a patient, especially on secondary findings. It may be impossible or impractical to fully inform certain patients on secondary findings with regards to genetics and inheritance probabilities. One piece of information can influence whether a patient understand the consequences or not. Patient

[17] "Bang v. Charles T. Miller Hospital Case Brief," 2014. http://www.lawschoolcasebriefs.net/2014/01/bang-v-charles-t-miller-hospital-case.html.

[18] Beauchamp and Childress, *Principles*, 88

understanding of genetics and biology varies considerably in the general population and there are people with an incredibly limited knowledge of these topics. Illiterate patients pose a standard problem for researchers looking to obtain informed consent and must rely on the practitioners to adequately get information across to these patients. It may also be impossible to fully inform patients in a philosophical sense where a patient cannot fully understand the process but this extreme notion is merely speculation. Patients benefit more from having knowledge available to them and those conducting research are ethically responsible for attempting to fully inform patients. While patients may not have the necessary experience to use the data themselves, there are experts in the field of genetics and medical clinics devoted to the interpretation of genomic information.[19] Genetic counselors are specialists devoted to the interpretation genomic data and clinical care of afflicted patients. Patients should be told of how to approach further genetic testing if they so desire from either the research consent forms or the clinicians. One targeted genetic test is not comprehensive, even with the inclusion of a secondary findings report. Because these reports are only the shallowest probe into problematic mutations, they should not be the end all for genetic testing which is why patients should be presented with options where they can bring the information they received from the initial genetic test.

Patients have different priorities and perspectives when they seek genetic counseling. One of the oft cited concerns of patients is privacy with regards to their genomic

[19] Olson et al, *Integrating*, 7-10, 18-19.

information.[20] One aspect of informed consent is about being within legal boundaries and knowing laws that affect patients and researchers. Current cultural movements have emphasized privacy and genetic data is known to be an identifier concerning some patients. In 1983, the US President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research prioritized confidentiality of genetic data over that of autonomy.[21] While NGS technologies have made it much easier for more people to be sequenced in recent years, the subject of ownership of genetic data and privacy has only been touched upon by the United States Federal government. In May 2008, the United States legislative branch passed the Genetic Information Nondiscrimination Act (GINA) guided by the Office for Human Research Protections and the Department of Health and Human Services. GINA prohibits discrimination of an individual in health insurance and employment based on genetic information.[22] Health insurers are restricted from requiring genetic information from an individual or their family as well as restricts using genetic information for coverage or policies. Employers are also restricted in the same manner where they are prohibited from requiring genetic information in all aspects of employment and may not terminate, hire, or influence promotions based on genetic information.

One area that is heavily influenced by GINA are the reporting of secondary findings. Clearly health insurance companies have a lot to gain from using genetic information in

[20] Olson et al, *Integrating*, 21.
[21] Lunshof and Ball, *Our Genomes Today: Time to Be Clear*
[22] OHRP, and DHHS. "Guidance on the Genetic Information Nondiscrimination Act: Implications for Investigators and Institutional Review Boards" March 24 2 (2009): 7.

making a decision on whether to insure to cover a patient who may be susceptible to hereditary diseases. Clearly, again, this poses an enormous issue for patients seeking health insurance and the patient-researcher relationship is the topic of this thesis. One issue in genetics testing is that mutations in certain genes are not guaranteed to lead to issues. These variants of uncertain significance are not guaranteed to be problematic so even though a patient has a mutation in that gene, it may not be deleterious or problematic. The understanding of deleterious mutations is still far too underdeveloped to be used as a deciding factor in insurance or employment. Employers may discriminate against employees or applicants because of their susceptibility to hereditary diseases which could take them out of workplace earlier than the employer desires. Secondary findings that would affect insurance or employment would be a huge concern for anybody obtaining genetic testing and would likely lead to more people rejecting these findings which are important for the future health of an individual. GINA is praised as being a baseline of protection against discrimination from genetic testing, however this law does not apply to employers with fewer than 15 employees, the military, and does not cover long term coverage, life insurance or disability insurance.[23] Knowing the legal situation surrounding genetic information may be important for patients seeking genetic testing which may discourage them from getting secondary findings. Using genetic information to discriminate against individuals is contentious because the current knowledge of genetics is not definite and many connections

[23] "Genetic Discrimination and Other Laws." *National Human Genome Research Institute*, 2014. https://www.genome.gov/10002077#al-3.

need to be described between genes. A mutation of unknown significance found on the BRCA2 gene may be benign but would still show up in a secondary findings report which a life insurance company may use as a determining factor in their coverage decision.

From an ethical perspective, discrimination based on genetic information disadvantages people based purely on what they have inherited. Similarities can be drawn to the discrimination based on race where both are aspects of a person's life that they never controlled. Knowing an individual's genetic information would be beneficial for the business practices of insurance companies and employers and there are still some loopholes for companies to use genetic information against people. Insurance and employment are two essential commodities in the contemporary United States that would likely be in jeopardy if GINA were not enacted. While this bill is a step in the right direction, GINA is only considered a minimum basis for protection of genetic information and many states have their own laws regarding genetic privacy that research institutes must be aware of.[24] More protections should be given to people based on their genetic information. In the case of genetic information protection from federal law, all information that is pertinent to the patient needs to be relayed. Patients will need to have enough information to make an autonomous decision and should know the exceptions to GINA. Certain exceptions may apply to a patient and it is up to the patient to decide whether they would like secondary findings reported; even if it means jeopardizing their chances of insurance or employment

[24] OHRP and DHHS, *Guidance*, 1.

because of current laws. If they are employed by a small business with less than 15 people or disability insurance important to their livelihood or family, they should be informed that their genetic information is not guaranteed to be protected under federal law. The cost-benefit of reporting secondary findings in that kind of scenario may be too great even if finding out future problems would be beneficial for them. Federal law is the depth that institutes like RG can inform the patient of whereas the clinician or genetic counselor will be more familiar with state laws regarding the privacy of genetic information. The clinician and researchers both provide an important contribution when it comes to giving the patient enough information for them to access their situation and make an autonomous decision.

The GINA definition of a genetic test includes analysis of human DNA, RNA, chromosomes, proteins or metabolites as well as tests detecting mutations, genotyping or chromosome changes.[25] Clearly this applies to genetic testing for hereditary diseases that patients come to Rare Genomics for. Patients should be informed of the laws that apply to genetics testing which is different for every state, however this is difficult for national research institutions. This is best left for clinicians and the genetic counselors who have direct, face-to-face contact with the patient. At the minimum, it is a responsibility of the researchers to inform patients of federal laws that apply to their genetic information.

A quick summary of GINA and the exceptions of this law should be discussed with the patient before genetic sequencing or in a release form; both for informed consent and

[25] OHRP and DHHS, *Guidance*, 2.

providing autonomous choice. In a study done by Simon et al. on disclosing individual research results (IRR) and informed consent processes, only 10 of a sample of 45 documents on IRRs and secondary findings included references to GINA.[26] If researchers do not reference the legal protections of a patient within the informed consent process, then veracity is lost. Veracity is essentially the respect for a patient's autonomy and a sense of justice. Researchers have an obligation from their position of power to act in the best interests of the patients which is bolstered by a sense of veracity. This obligation being comprehensive, accurate and objective transmission of information to the patient. The professional also fosters an understanding in the patient and this obligation is only non prima facie when it conflicts with other obligations that the professional has. Information is prima facie with patients but this may be complicated if patients are deemed incapable of handling certain information. The hypothetical situation here would be if anxiety is a serious factor in fully informing the patients of secondary findings. Presumably patients would desire to hear the secondary findings report after they have made the autonomous decision to have them reported with the genetic test, however they may change their mind in the interim period or prove incapable of handling information. Should researchers find deleterious mutations in the list of scanned genes that could prove serious, there is an argument to be made for informing the family but keeping the information from the patient. This choice; possibly

[26] Simon, Christian M., Laura Shinkunas, Debra Brandt, and Janet K. Williams. "Individual Genetic and Genomic Research Results and the Tradition of Informed Consent: Exploring U.S. Review Board Guidance" 38, no. 7 (2012): 417–22. doi:10.1136/medethics-2011-100273.

recommended by researchers, and made by clinicians draws a thin line between disrespecting autonomy and the principle of beneficence. This is likely a case of an ethics of care and specification where context is incredibly important in these situations. Another situation where clinicians should care about context is the difference between express and tacit consent. Express consent is outright making a decision and tacit consent is consent by a lack of objection.[27] Researchers essentially demand express consent by asking patients to read and sign release forms and tacit consent should not be an issue for researchers. After reporting the secondary findings to the clinician is where tacit consent may arise when a patient may be unsure of reading or hearing about the report themselves.

Informed consent with regards to human genetics research can only be achieved if the patient has fully understood the information disclosed by consent documents and clinicians. This is generally used to consent to the research or procedure that is to be done but the informed position may go both ways. An individual may refuse to participate in genetic testing based on informed refusal where they understand everything that is to be done but make the conscience decision to not participate. Informed refusal is not the goal of researchers trying to inform patients but they must respect the decision made by the patient based on the principle of autonomy discussed later. Informed refusal does not include the possibility of false beliefs or misinterpretations. An example of informed refusal with regards to secondary findings could include a patient who understands what genetic testing can offer

[27]Beauchamp and Childress, *Principles*, 65

them but, going back to the exceptions of GINA, values disability insurance that could be jeopardized by the secondary findings of the test. Patients should not have to fear the discrimination of insurance companies or employers however there are exceptions under federal law that may apply in this patient's situation. Researchers have to respect informed refusal in secondary findings, even if the patient's agree to topical genetic testing like looking for a gene that is correlated to the patient's phenotype. Related to informed refusal but not as disheartening is the possibility of patients to waiver the process of informed consent. Clinicians would get the waivers from the patient's which relives the clinician of having to consent as the patient just consents. The patient loses the ability to be informed in this case but this situation is ethically and legally risky for researchers and clinicians. RG requires patients to provide their complete medical records and the sequencing files of the patient and immediate family members so relinquishing informed consent is made harder through release form processes.[28] Adding an option for secondary findings may allow patients to just sign at the dotted line without understanding the benefits and risks they are taking. Release forms should try and mediate relinquishing informed consent because it does not benefit the patient to the degree that an informed decision would. Few approaches respect the autonomy of the patient so the best approach would include a section reminding the patient to ask questions they may have regarding secondary findings and include a smaller line for the initials of the patient. It is a right of the patients to be informed when they decide on tests

[28]Institute, Rare Genomics. "Authorization for The Release of Medical Information," n.d.

that affect their future and while it is a right of them to also waiver informed decision making, it is not recommended for optimal benefit.

The benefits of individual patients extend to the general population when included in genomic data pools for research use. Data sharing in biobanks is a growing practice by government, research institutions and clinics with the purpose of advancing research for personalized and preventative medicine. The National Institutes of Health issued the Genomic Data Sharing Policy in 2014 which dictates that all genomic data collected for research funded by the NIH will be collected within a biobank. This allows scientists to use data that has already been collected and expedite the creation of knowledge.[29] Other research that benefits from biobanks are studies on association between genotypes, socioeconomic parameters, environmental exposure and phenotypes.[30] The express goal of the NIH is to make genomic data publicly available while also protecting the data and privacy of individuals. The issue of biobanks is the publicity of data and the unique genetic sequence that all individuals have. States now employ biobanks with newborns for identification purposes making total genetic non-disclosure essentially invalid. Patients may have questions or concerns regarding their identity and privacy if their data is included in a biobank. RG is not inclined to follow NIH statutes and this problem is less relevant for this institute, however biobanks are a concern for some patients and researchers. Debates surround

[29] "GENOMIC DATA SHARING (GDS) Home." *U.S.Department of Health & Human Services*. Accessed February 14, 2016. https://gds.nih.gov/03policy2.html.

[30]Gurwitz, David, Isabel Fortier, Jeantine E Lunshof, and Bartha Maria Knoppers. "Children and Population Biobanks." *Science* 325, no. August (2009): 818–19. doi:10.1126/science.1173284, 818

biobanks and how they could be used against patients or individuals but this is not relevant to the purpose of this thesis. I will however address the issue in brief. Biobanks are not something to fear for patients because it is not unlike government social identification and records. Non-invasive government policies already affect all individuals that are likely to be associated with the genetic sequencing process. With the express goal of the NIH being the privacy of the genetic data collected for some research, there is little concern for the abuse of genetic information.

Research institutions need to make these documents readable and available to patient's; providing the options for patients to act autonomously and voluntarily while also acting ethically and beneficently. The FDA's regulations on informed consent require that information presented to the patient should be in language understandable to the patient. No language should be used where the patient must waive or appear to waive any legal rights or release the institution from liability in the case of negligence.[31] The language of release/consent forms are very important for all parties involved. The forms must be concise yet informative and readable which is sometimes difficult when discussing genetics and medical jargon. While a majority of researchers, research participants and patients desire to report secondary findings, studies suggest that research participants and patients receive

[31] U.S. Department of Health and Human Services, Food and Drug Administration, Office of Good Clinical Practice, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, and Center for Devices and Radiological Health. "Informed Consent Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors," 2014, 5.

more complex information that they can reasonably assimilate and use in decision making.[32] The assumption made is based on the reasonable person standard which assumes that information is disclosed to a hypothetical rational and reasonable person. This is a best case scenario when informing patients and their families but this cannot be true in all cases. Sometimes the patient is a non-autonomous individual like a child whose parents or surrogates do not have the ability to comprehend the ramifications of analysis and secondary findings and may not be able to read consent forms. Information needs to be tailored to that individuals needs which is difficult to achieve when institutions like RG are separated from the patient. The clinician or genetic counselor has the best ability to tailor the information to the patient so it is recommended that the patient consult their care provider before making decisions. For the part of the researchers, information given to the patient should be in language that is understandable to the general population. Simon et al. analyzed the average grade level readability of language found in reports and forms given to patients and found that the average grade level found in analysis given to patients ranged from 9-12.[33] This is a fair range for most people with a high school diploma but not everyone who may be a patient or decision maker has that accomplished. The best thing for research institutions to do would be making their forms as understandable as possible which can be a difficult feat to achieve. Because of the difficulty of the language and topic, it would be best if the patient was to consult their clinician or genetic counselor before making a decision.

[32] Appelbaum, *Models of Consent*, 19
[33] Simon et al, *Individuals*, 7.

Clinicians will often be the ones to consult the patients and disclose the information provided by the research institutions but there will need to be a combined effort between the clinicians and researchers in obtaining autonomous consent from patients. The method for contact and consent of patients for the research institution is in-depth consent forms. These medical forms are the basis for communication between researchers and patients. The problem that patients may experience is processing the amount of information given to them and how to respond to the data. The medical release form for RG that relinquishes the genetic and medical information to researchers is sent directly to the patient who then sends the form back to the institute. There is no contact between patients and medical professionals like clinicians or genetic counselors who could help inform patients about what they are giving to the institute and what they can expect in requesting secondary findings. Researchers have a duty to inform patients of what they will be analyzing and what the patient can expect but there is only so much that communication with a representative and a three-page form can do in informing patients. It is unreasonable to expect that a patient will be fully informed with this information, especially when including a secondary findings request section in the medical release form. If the patient has questions regarding medical information or secondary findings, they must contact the clinician or counselor themselves which is a difficult thing to expect a patient to do. In the document, I recommend that patient's contact their genetic counselor if they have questions about secondary findings but this is only a recommendation and leaves a lot to be desired. If at all possible, it is recommended that the research institution go through genetic counselors or clinicians when

giving patients the medical release form. While sending the medical release form directly to patients via email expedites the process for analysis, the expectation of informed consent is unrealistic. If there is no change to this process it is still acceptable to send the medical release and secondary findings request to patients because we have given them the autonomous choice to be fully informed.

Autonomy

The underlying idea central to the thesis so far is the notion of autonomy. Autonomy is a large focus of the issue on secondary findings and the respect for patient's autonomy and practicality sometimes conflict. Not surprisingly, many ethical issues arise when discussing autonomy and secondary findings. The patient has overriding authority on the conditions of their genomic data, but as is oftentimes the case with Rare Genomics patient's, there is surrogate decision maker because the patient is unfit to make their own autonomous decisions. These patients could be infants or children or unable to consent because of their hereditary or mental illness. The clear ethical standard for researchers is the beneficence of the patient. The substituted judgement standard, where a surrogate makes decisions on what the patient "would have made",[34] and the pure autonomy standard, where surrogates respect the wishes of the formerly autonomous,[35] do not usually apply in these situations and tend to

[34] Beauchamp and Childress, *Principles*, 99
[35] Beauchamp and Childress, *Principles*, 100

be sub-par ethical compromises. Researchers need to adopt a best interest standard when it comes to secondary findings and patients.

Autonomy is described as being a decision free of outside influence and coercion. This is meant as the patient making a voluntary decision to participate in the research without acting from another's influence. The focus is on the decision of the patient rather than the outside circumstances that influence the decision like finances or personal relationships. Sometimes this is not the case as described above in the case of children or the mentally ill. There is occasionally the need for surrogate decision making which is defined as someone making decisions on behalf of those not capable of making informed decisions.[36] Parents are the surrogate decision makers for children and are expected to act in the best benefit of the child. Parents also have the autonomous decision of whether or not to include secondary findings within the test reports. This is sometimes complicated when the decision to act is based on personal beliefs which are not always in the child's best interest. The famous common example of this is the parents of Jehovah's Witnesses who decline to give their child a lifesaving blood transfusion because of their beliefs. The issue connected with secondary findings would be the cost-benefit of getting secondary findings reported. While not as drastic as a blood transfusion, there is a clear benefit for the future of the child if they were to know things that could affect them in the years following the initial genetic testing.

[36] Beauchamp and Childress, *Principles*, 99

The child itself cannot express consent but may decide that it would like access to the secondary findings report once they become autonomous individuals. Many issues arise between the future presumed autonomy of the child, the surrogate authority of the parents, and reporting of secondary findings. This relationship is complex in that there is the potential harm of labelling a child as bound to develop problems in the future (e.g. increased chance of certain cancers) contrasted with the benefit of knowing early enough to possibly plan or commit to treatment. There is also the need to protect the privacy of the child and their genetic information.[37] The problem is further complicated by the anxiety that parents may experience at having transferred diseases to their child or discovering non-paternity through analysis. Solutions to these problems are incredibly contextual and involve researchers, patients, surrogates and clinicians alike. Should the parents deny reporting of secondary findings via informed refusal, then that leaves the child at a loss of information later on in life. If researchers conduct secondary finding reports on children and save them, the surrogate authority of the parents is challenged and the medical professionals are responsible for holding the information and getting it to the child when they come of age. The question lies with what are the child's right to know with their own genetic information. The unfortunate reality is that some children will never be capable of autonomous decisions and in this case, the parents have the overarching authority for decisions. Fortunately, this is not every case and there are children that have the capability

[37] Clarke, Angus J. "Managing the Ethical Challenges of Next Generation Sequencing in Genomic Medicine." *British Medical Bulletin* 111, no. 1 (2014): 17–30. doi:10.1093/bmb/ldu017, 25

to become autonomous even with hereditary disadvantages. In the interest of giving the child choice in the future, genetic testing institutions should allow these children to receive their secondary finding reports or offer to reanalyze the data when the child comes of age. One option is to have a preliminary list of genes to scan and save this secondary findings report. Another option is to offer to retest the child when they become autonomous. The latter option is attractive because it provides more time for genetic information technology and knowledge to grow, providing a more wholesome picture. Variants of unknown significance may be studied more in depth so researchers will know more about how mutations affect gene function. This assumes the institute will remain operational for a long period of time which may not be the case when the child becomes autonomous. Even though there is a chance of the institute discontinuing operations before the child becomes autonomous, NGS technologies are making genetic testing cheaper and cheaper.[38] Presumably genetic testing will be more affordable in the coming years making the loss of reanalysis by one institute near inconsequential. Research institutions like RG should offer reanalysis of secondary findings when the child becomes autonomous. Some companies already offer this for patients.[39] The issue is null provided parents opt for the inclusion of secondary findings for the child.

Things are further complicated when it is not the parents who are designated the surrogate authority or the situation is unclear. The spouse, child, family member or maybe

[38] Olson et al, *Integrating*, 1
[39] Stephanie Gurnon, former genetic counselor, in a conversation with the author, February 2016.

even a close friend may be the surrogate decision maker for an individual and clinicians should be careful when accepting decisions from surrogates. Claims about what patients supposedly want are inherently dubious coming from secondary parties. The relationship to the patient and the clinician's opinion should be considered when considering the qualifications of the surrogates. Researchers are mostly separated from the patients for the sake of anonymity so the clinician's viewpoint is an important factor in this case with decision making. Notions of the rules of surrogate authority and the rules of professional authority in ethics surround the issue of non-autonomous patients.[40] Both of these rules serve to guide who should serve as a surrogate agent to the patient and when a professional has the right to override a patient's decisions if they are harmful or poorly contemplated. A quality of life standard is usually adopted where a surrogate makes the decisions in the patient's and the family's best interest. The issue here is that the surrogacy dynamic sometimes clashes with the professional opinion of clinicians or researchers. Researcher's concerned over the decision made about the patient have little effect on the decision making regarding secondary findings. Even given multiple important findings that could affect the patient adversely, if the patient or surrogate agent declines including these findings through informed refusal, the researcher must respect the autonomy of these patients. Something a researcher may do is look for these secondary findings save them in case the patient decides to revisit the findings later or give them to the patient's clinician to file away should they have a moral and ethical defense for doing so.

[40] Beauchamp and Childress, *Principles*, 13

Patients need only a substantial understanding of affairs for the decision to be considered autonomous. A patient may act autonomously without exercising their right to information. A patient who signs the form authorizing secondary findings without reading the information provided is still qualified to act autonomously but chooses not to do so.[41] Examples like this bring up the debate of what autonomous people want vs. what they should want. For secondary findings, it is easy to assume that the logical patient would want as much information available to them so they can make the best autonomous choice. This is not always the case for example, patients may fear learning of other problems they may experience or may not care to learn any more than they have to. People are attracted to medicinal clinics because they provide professionals qualified to deal with diagnoses and disease. People are directed to institutes like RGI because these places offer more information analyzed by qualified individuals that benefit the patients. Clearly patients wish to be informed but generally agree to have the medical professionals guide them and make decisions.[42] With this in mind, some patients may ask clinicians to make the decision for them believing that they are more informed and will make the best decision on their behalf. Autonomy is a right of the patient and it should not be confused as a duty of the patient for this puts undue pressure onto patients. Researchers cannot force people to make decisions they do not want to make. Making patients delegate on every issue when they have made it clear they wish to delegate that responsibility elsewhere is a clear violation of autonomy.

[41] Beauchamp and Childress, *Principles*, 59
[42] Beauchamp and Childress, *Principles*, 61

While the medical professionals should not push the patients to decide everything, in a standard clinic there is a clear risk of clinician bias towards certain methods of operation that may not actually be in the best interest of the patient. In the case of genetics testing and in order to provide the most information possible to the patient for future use, if a clinician or researcher is given the choice for the autonomous patient then they should have the secondary findings reported and stored with the clinician for the patient's benefit. Having the information accessible to the patient who may be unsure of how to address secondary findings at the time or in the future gives the patient the most options to work with. The act of patients passing on their autonomous decisions to those they view as more qualified should clearly benefit them and having options gives the patient considerable freedom with their genetic information. This is in line with the notion of mutual decision making between the patient and the qualified professional discussed previously. The medical professional has too much power over information and the patient may not know what they are signing up for. Researchers and clinicians should advocate for reporting secondary findings to patients but should remain conscious of the patient's overarching autonomy.

The approach that is described to benefit the patient with regards to autonomy is choice. Giving options to patients is the best approach to an informed consent policy regarding human genetics research and secondary findings. In a study that analyzed the many informed consent forms around the western world given to patients, it found that around one third of the documents provided a clause for whom researchers were able to give

information to regarding the individual research results (IRR) and incidental findings (IF, also known as secondary findings). The options given were the patient's doctor, a spouse or family member and other.[43] Documents like this tackle two problems by establishing autonomy of the patient through choice and giving solutions to where secondary findings may be sent after analysis. Rather than asking the patient to give a binary answer of whether they would like to receive secondary findings, they are given the chance to evaluate where they would like the information retained before testing. Information overload for patients is a challenge that researchers face when obtaining consent. The diversity of patient's knowledge of genetics and biology may lead to issues where an individual does not have the capability to fully understand what is being asked of them and how to interpret the data. Oftentimes the mode of presenting risks or consequences to patients has an outcome on their decisions.[44] Rephrasing certain terms like "secondary findings" to things such as "other findings" may have a large impact on if a patient will consent to the research and if they would be willing to receive secondary findings. Similarly, expressing probability in numeric and non-numeric means and the use of analogies also helps the patient as well as opening up the possibility of more people having a better understanding secondary findings. Using simplified terms to get across the importance of secondary findings and the research itself is invaluable in consent forms sent to patients and their families. An example of this would be providing metaphor examples for patients to better understand genetics jargon. Providing

[43] Simon et al, *Individuals*, 7.
[44] Beauchamp and Childress, *Principles*, 90-92

examples of how secondary findings have affected people and diagnoses may also provide the patients with a sense of how genetics testing and these findings are important.

What Should Be Included in a Secondary Findings Report?

Informed consent and autonomy are given ethical values that benefit both the patients and researchers. Within the chapters on informed consent and autonomy, vague mentioning of certain aspects of secondary findings reports occurred. An example would the section on how to dissuade patients from relinquishing informed consent. The purpose of this chapter is to summarize what and how information should be included within secondary findings reports with the focus on the inner workings of the Rare Genomics Institute.

In order to discuss the method of presenting secondary findings to patients, RG operations need to be explained. The Rare Genomics Institute operates as a non-profit organization designed to help diagnose and research rare diseases around the world. The goal is to help patients with finding personalized research projects and diagnoses.[45] The patient or the patient's family contacts RG usually with the guidance of a physician and are then sent an application and medical release form. Patients are assigned a Patient Advocate who is the contact between the patient and RG and helps guide them through the exome sequencing and analysis process. RG then connects the patient with a clinical geneticist who then

[45] "How We Help Patients." *Rare Genomics Institute*, 2016. http://www.raregenomics.org/process/.

determines the eligibility of sequencing and what tests would be beneficial to have beforehand. After being approved for testing the results take around 2-6 months to be report back with between 1/3 and ¼ of patients receiving new diagnoses. After initial testing, patients who desire further analysis of the raw data are dealt with by the RG Science 2.0 team. The most desired outcome is that a gene is identified that is likely causative of the patient's problems and RG recommends possible treatment or research options for the patient to pursue. Some patients are reluctant to commit to testing for various reasons including monetary ones. RG helps families crowdfund their sequencing by the recommended clinical geneticists through posting the story of the patient on their website.[46] The process of analysis begins after the director of RG's Science 2.0 team accepts the case. The cases are passed to volunteer analysts who complete a report which is then edited by the director of the team. A case will only be submitted if it has been reviewed by RG's global network of experts, the analyst and a Community Manager. Once this has been completed, the director of the Science 2.0 team edits the final report and sends it to the family.

The argument of this thesis is that a secondary findings report should be included within the final report given to the patient. Currently RG does not deal with secondary findings so the list of genes on the report are only the ones that pertain to the phenotype and medical history of the patient. In order to sign up for genetic testing and analysis of results through RG, patients must fill out a three-page medical release form which asks for the

[46] "Donate Directly to Rare Genomics Patients." *Rare Genomics Institute*, 2016. http://www.raregenomics.org/patient-donations/.

complete medical records of the patient and the genetic sequencing files of the patient and immediate family members. Bolded are the words; TYPE OF INFORMATION REQUESTED and below the two requested items is a section on patient rights and cancellation notices. The patient's rights state that the signatory has the right to revoke or cancel the medical release authorization at any time in writing. The cancellation notice section relieves RG of the liability of information being released before the receipt of the written notice for cancellation.

TYPE OF INFORMATION REQUESTED

Complete medical records of patient

Genetic sequencing files of patient and immediate family members

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PATIENT RIGHTS

You have the right to revoke or cancel this authorization, in writing, at any time

CANCELLATION NOTICE

Rare Genomics Institute will not be held responsible for any release of medical information accomplished before receipt of a written notice cancellation. Revocation takes place from the date of receipt of written request.

Figure 2: Section of the Rare Genomics Institute's medical form given for visualization.[47]

This small section of the medical release form is an optimal place for the secondary findings request and subsequent relevant information. It is placed just below what is being requested

[47] Institute, Rare Genomics. "Authorization for The Release of Medical Information," n.d.

via the medical release form and just above the instructions for release cancellation. The patient will see both the summary of what they will receive, their rights and what course of action they may take should they change their mind about genetic testing. The wording for a section designed to ask whether a patient wants secondary findings to be reported should be similar to the legal language of the medical release form. As discussed on pages 25, it should contain two signatory sections so that a patient will be more encouraged to be informed and not relinquish their right to informed consent. The section itself should have a request for whether the patient would want a secondary findings report to be included with the results of the Science 2.0 team's analysis, as well as instructions for who is to receive the report if not just the patient. Included under the question with a yes or no checkmark should be a brief definition of secondary findings, a suggestion to talk to a trusted clinician, and the legal rights of a patient.

This section should absolutely be separate from the medical release request information because it is more important that the patient understand that secondary findings are optional which is why it should include a separate signatory section. Similarly, a short blurb on the Genetic Information Non-Discrimination Act is included in the section on "Patient's Rights" as discussed in the *Informed Consent* chapter. An example of what the medical release form should include is written below.

AUTHORIZATION FOR THE RELEASE OF MEDICAL INFORMATION

INFORMATION TO BE RELEASED BY

Name | *Relationship to patient* | *Email*

Street address | *City* | *State*

INFORMATION TO BE RELEASED TO

Organization: Rare Genomic Institute

Address: 2657 Annapolis Road, Suite G #105, Hanover, MD 21076

Contact name: Romina Ortiz

Contact Email: romina.ortiz@raregenomics.org

TYPE OF INFORMATION REQUESTED

Complete medical records of patient

Genetic sequencing files of patient and immediate family members

*Newly Added Section

Secondary findings are defined as being medical information that is discovered unintentionally and is unrelated to the condition being tested. These would be diseases that the patient is susceptible to develop in the future. We recommend you discuss the implications of these findings with your doctor before agreeing to the secondary findings.

For more information on secondary findings, please visit this website:

(http://bioethics.gov/sites/default/files/Clinician%20Primer%20-%20FINAL.pdf)

Would you like to include a secondary findings report in addition to the analysis report for the condition being tested?

__ Yes __ No

If you would like to delegate who should receive these findings, please contact your Patient Advocate.

PATIENT RIGHTS

You have the right to revoke or cancel this authorization, in writing, at any time

*Newly Added Section

You are protected under federal law by the Genetic Discrimination Non-Discrimination Act to not be discriminated against because of your genetic data by employment or health insurance companies. This law does not apply to employers with fewer than 15 employees nor disability or life insurance policies. Local and State laws vary on added protections; we recommend you discuss the legal implications of genetic testing with your clinician or genetic counselor before submitting this form.

CANCELLATION NOTICE

Rare Genomics Institute will not be held responsible for any release of medical information accomplished before receipt of a written notice cancellation. Revocation takes place from the date of receipt of written request.

I understand that the information I give is my own submission of medical tests, treatments and results pertaining to myself, my child, and immediate family.

I hereby consent to the release of the specified information I am giving through the medical-records sharing website Patients Know Best and allow you to use it only for research purposes including sharing de-identified information with external experts and

phenotype/genotype databases. I understand that such information cannot be released without my informed consent. I acknowledge I have fully reviewed and understand the contents of this authorization form. My signature below indicates that I hereby agree to and authorize the release of patient health information to Rare Genomics Institute.

Name *Signature*

Date

Instructions for Canceling a Request

You must provide a written request to romina.ortiz@raregenomics.org for revocation/cancellation of the original record release.

We need to have your complete name, date-of-birth, telephone number (home/work) and the name of the person/agency that you authorized to receive the medical information.

After receipt of the notice, email confirmation will acknowledge your withdrawal of authorization.

If you have any questions concerning the cancellation process, email. romina.ortiz@raregenomics.org

Figure 3: Recommendation for the secondary findings addition to the medical release form.

There is room for volunteer analysts to add on a secondary findings report from the test results onto the final report. The time it takes to check a list of around 65 genes would only be a small part of total time taken for analysis. Providing filters for genes targeted to be reviewed by analysts are already a part of many databases like Omicia Opal. Adding filters for secondary findings reports streamlines the process and allows analysts to make the call over whether a mutation is significant enough to report or not. As discussed earlier, there are

concerns over mistakes about the significance of the gene. In the case of these genes, they tend to be monogenic disorders that have been studied extensively and have been shown to be pathogenic. With the use of Clinvar, COSMIC and Omicia Opal, these genes have been determined to be problematic for individuals; without necessarily expressing a clear phenotype of the disease. Omicia has an indicator of the likelihood of deleteriousness of a mutation which is a good start to analysis. The genetic information loaded onto Omicia is analyzed by the database which shows indicators of deleteriousness as well as what kind of mutation occurred. The template genome comes from the 1000 Genome Project and Omicia compares the subject's genome to this collection to determine what has been mutated.

RG's genetic analysis is not supposed to be the end all with genetic sequencing. One issue that should be made clear within the language of consent forms is that these secondary findings are not a final diagnosis. Often mutations within certain genes just increase the likelihood of developing the disease that has been associated with that gene. RG's analysis of genetic data is not entirely comprehensive for there are other problems that can be identified from the genome that RG does not look for. There is a lot of information to be analyzed from whole exome sequencing and we do not currently have the capability of determining everything that is possibly disease causing. Included in this thesis are carrier status genes, primarily X-linked, that are mutated but do not affect the carrier of the mutated gene. The reason for reporting these genes is to inform the patient or immediate family members that they have a possibility of passing on this mutated gene to future offspring which could be

deleterious. An example of this is Fabry's disease which is a recessive lysosomal storage disease that is found on the X chromosome and affects all males with the mutation as well as affects homozygous and some heterozygous females. Informing patients that they are a carrier for this disease can affect the future decisions of said patient and may help diagnose other immediate family members with the same mutation. There are far too many of these kinds of diseases for one secondary findings report to cover. RGs secondary findings report would cover some of the most important diseases to date with room for improvement in the future. For a complete analysis of what parents could pass on to their offspring, they will need to approach another genetic analysis opportunity. RG is devoted to figuring out the cause of a patient's suffering and preventing future cases of the disease through individualized care and they cannot possibly be the end-all for any genetic disease that a patient may have.

Content of Secondary Findings Report

The meat of any secondary findings report are the genes that are found to be significantly mutated and likely deleterious. The following genes come from both the American College of Medical Genetics and Genomics list of recommended genes and research of monogenic disorders. This section goes through the ACMG list in alphabetical order by gene. The end will be genes that I recommend be added to both this list and to secondary findings reports which are listed as new recommendations. The list is kept short

because RG testing is not an end-all of genetic testing and there is more to be learned about certain genes which may be determined to be deleterious.

The table is organized alphabetically by gene and includes the disease that the gene is associated with, the inheritance pattern, disease type and recommender. The disease is hopefully explained a little bit by the disease type which is just a basic category or consequence of having the deleterious gene. The legend shows the acronyms for the inheritance patterns found on the list. One may refer back to the chapter *An Introduction to Genetic Analysis* for more information and a refresher on some of the terms used in the upcoming section. The table is designed to reflect the inheritance of the disease that is included on the list with subsequent inheritance (separated by a hyphen) that are in the same row represent the inheritance of other diseases that the gene is associated with.

Legend	
AD = Autosomal Dominant	XL = X-Linked
AR = Autosomal Recessive	XLR = X-Linked Recessive
Smu = Somatic Mutation	XLD = X-Linked Recessive
Mu = Multifactorial	? = Unknown Inheritance

Gene	Disease	Inheritance	Disease Type	Recommender
ACTA2	Aortic aneurysm, familial thoracic 6	AD	Heart Disease	ACMG listed
ACTC1	Familial hypertrophic cardiomyopathy 11	AD	Heart Disease	ACMG listed
APC	Adenomatous polyposis coli	AD	Cancer Susceptibility	ACMG listed
APOB	Familial hypercholesterolemia	AD - AR?	Elevated Cholesterol	ACMG listed

Gene	Disease	Inheritance	Disease Type	Recommender
ATRX	Alpha-Thalassemia/Mental Retardation Syndrome	XLD	Mental Retardation - Carrier status	New Rec.
BRCA1	Breast-ovarian cancer, familial 1	AD	Cancer Susceptibility	ACMG listed
BRCA2	Breast-ovarian cancer, familial 2	AD	Cancer Susceptibility	ACMG listed
CACNA1S	Malignant hyperthermia	AD - ?	Skeletal Muscle Disorder - Volatility to Anesthesia	ACMG listed
CFTR	Cystic Fibrosis	AR	Carrier status	New Rec.
COL3A1	Ehlers-Danlos syndrome, type 4	AD	Proneness to Rupture of Arteries	ACMG listed
DMD	Duchenne Muscular Dystrophy	XLR	Progressive Proximal muscular dystrophy	New Rec.
DMD	Becker Muscular Dystrophy	XLR	Progressive Proximal muscular dystrophy less severe	New Rec.
DSC2	Arrhythmogenic right ventricular cardiomyopathy, type 11	AD	Heart Disease	ACMG listed
DSG2	Arrhythmogenic right ventricular cardiomyopathy, type 10	AD	Heart Disease	ACMG listed
DSP	Arrhythmogenic right ventricular cardiomyopathy, type 8	AD	Heart Disease	ACMG listed
FBN1	Marfan's syndrome	AD	Connective Tissue Disease - Dominant Negative	ACMG listed

Gene	Disease	Inheritance	Disease Type	Recommender
FMR1	Fragile X syndrome	XLD	Mental Retardation - Carrier status	New Rec.
GLA	Fabry's disease	XL	Heart Disease and Renal Failure	ACMG listed
HTT	Huntington's Disease	AD	Progressive Neuronal Degeneration	New Rec.
IDS	Mucopolysaccharidosis type II	XLR	Progressive Degeneration	New Rec.
IKBKG	Incontinentia Pigmenti	XLD	Carrier status	New Rec.
KCNH2	Long QT syndrome 2	AD	Heart Disease	ACMG listed
KCNQ1	Long QT syndrome 1	AD - AR?	Heart Disease	ACMG listed
KRAS	Leukemia, acute myeloid	AD	Cancer Susceptibility	New Rec.
LDLR	Familial hypercholesterolemia	AD	Elevated Cholesterol	ACMG listed
LMNA	Dilated cardiomyopathy 1A	AD - AR?	Heart Disease	ACMG listed
MEN1	Multiple endocrine neoplasia, type 1	AD	Cancer Susceptibility	ACMG listed
MLH1	Lynch syndrome	AD	Cancer Susceptibility	ACMG listed
MSH2	Lynch syndrome	AD - AR?	Cancer Susceptibility	ACMG listed
MSH6	Lynch syndrome	AD	Cancer Susceptibility	ACMG listed
MUTYH	MYH-associated polyposis	SMu	Cancer Susceptibility	ACMG listed
MUTYH	Pilomatrixoma	SMu	Cancer Susceptibility	ACMG listed
MYBPC3	Dilated cardiomyopathy 1A	AD	Heart Disease	ACMG listed
MYBPC3	Familial hypertrophic cardiomyopathy 4	AD	Heart Disease	ACMG listed
MYH11	Aortic aneurysm, familial thoracic 4	AD	Heart Disease	ACMG listed

Gene	Disease	Inheritance	Disease Type	Recommender
MYH7	Familial hypertrophic cardiomyopathy 1	AD	Heart Disease	ACMG listed
MYL2	Familial hypertrophic cardiomyopathy 10	?	Heart Disease	ACMG listed
MYL3	Familial hypertrophic cardiomyopathy 8	AD	Heart Disease	ACMG listed
MYLK	Aortic aneurysm, familial thoracic 7	AD	Heart Disease	ACMG listed
NDP	Norrie Disease	XLR	Degenerative Blindness and Mental Capacities	New Rec.
NF2	Neurofibromatosis, type 2	AD	Cancer Susceptibility	ACMG listed
OTC	Ornithine transcarbamylase deficiency	XLR	Metabolic Disorder	New Rec.
PCSK9	Hypercholesterolemia, autosomal dominant, 3	AD	Elevated Cholesterol	ACMG listed
PKP2	Arrhythmogenic right ventricular cardiomyopathy, type 9	AD	Heart Disease	ACMG listed
PMS2	Lynch syndrome	?	Cancer Susceptibility	ACMG listed
PRKAG2	Familial hypertrophic cardiomyopathy 6	AD	Heart Disease	ACMG listed
PRNP	Gerstmann-Straussler Disease	AD	Prion Caused Progressive Degeneration	New Rec.
PTEN	PTEN hamartoma tumor syndrome	AD - AR?	Cancer Susceptibility	ACMG listed
RB1	Retinoblastoma	SMu - AD?	Occular Degeneration	ACMG listed
RET	Familial medullary thyroid carcinoma	AD	Cancer Susceptibility	ACMG listed
RET	Multiple endocrine neoplasia, type 2a	AD	Cancer Susceptibility	ACMG listed
RET	Multiple endocrine neoplasia, type 2b	AD	Cancer Susceptibility	ACMG listed

Gene	Disease	Inheritance	Disease Type	Recommender
RYR1	Malignant hyperthermia	AD - AR?	Skeletal Muscle Disorder - Volitility to Anestesia	ACMG listed
RYR2	Catecholaminergic polymorphic ventricular tachycardia	AD	Heart Disease	ACMG listed
SCN5A	Brugada syndrome 1	AD	Heart Disease	ACMG listed
SCN5A	Long QT syndrome 3	AD	Heart Disease	ACMG listed
SDHAF2	Paragangliomas 2	AD	Cancer Susceptibility	ACMG listed
SDHB	Paragangliomas 4	AD - ?	Cancer Susceptibility	ACMG listed
SDHC	Paragangliomas 3	AD - ?	Cancer Susceptibility	ACMG listed
SDHD	Paragangliomas 1	AD - AR?	Cancer Susceptibility	ACMG listed
SMAD3	Loeys-Dietz syndrome type 3	AD	Heart Disease	ACMG listed
SMAX1	Spinal and bulbar muscular atrophy	XLR	Muscular Degeneration	New Rec.
STK11	Peutz-Jeghers syndrome	AD - SMu, Mu?	Cancer Susceptibility	ACMG listed
TGFBR1	Loeys-Dietz syndrome type 1A	AD	Heart Disease	ACMG listed
TGFBR1	Loeys-Dietz syndrome type 2A	AD	Heart Disease	ACMG listed
TGFBR1	Marfan's syndrome	AD	Connective Tissue Disease	ACMG listed
TGFBR2	Loeys-Dietz syndrome type 1B	AD	Heart Disease	ACMG listed
TGFBR2	Loeys-Dietz syndrome type 2B	AD	Heart Disease	ACMG listed
TMEM43	Arrhythmogenic right ventricular cardiomyopathy, type 5	AD	Heart Disease	ACMG listed
TNNI3	Familial hypertrophic cardiomyopathy 7	AD - AR?	Heart Disease	ACMG listed

Gene	Disease	Inheritance	Disease Type	Recommender
TNNT2	Left ventricular noncompaction 6	AD	Heart Disease	ACMG listed
TP53	Li-Fraumeni syndrome 1	AD - AR, SMu?	Cancer Susceptibility	ACMG listed
TPM1	Familial hypertrophic cardiomyopathy 3	AD	Heart Disease	ACMG listed
TSC1	Tuberous sclerosis 1	AD - ?	Cancer Susceptibility	ACMG listed
TSC2	Tuberous sclerosis 2	AD	Cancer Susceptibility	ACMG listed
VHL	Von Hippel-Lindau syndrome	AD - AR?	Cancer Susceptibility	ACMG listed
WAS	Wiskott-Aldrich Syndrome	XLR	Autoimmune Deficiency - Carrier status	New Rec.
WT1	Wilms' tumor	SMu - AD, AR?	Cancer Susceptibility	ACMG listed

Similar characteristics found on this list are disease type and mode of inheritance. A lot of the genes recommended are associated with heart disease or cancer susceptibility. This pattern is representative of the severity of diseases that these genes cause. Heart diseases and cancer are highly lethal even with today's modern medical practices. One common trait that almost all of these genes share is that they do not display symptoms until later in life. Diseases like Familial hypertrophic cardiomyopathy and Familial medullary thyroid carcinoma do not present symptoms until 40 or 50 years into life, and one of these symptoms is sudden death. All of these genes are included because the patient may not know that they are susceptible to these diseases at the time of genetic testing. Many of these diseases show autosomal dominant inheritance meaning people have a roughly 50% chance of obtaining

the mutated and problem causing gene from their affected parents. The severity of the disease and the mode of inheritance are also primary factors in what is included on this list, for both the ACMG recommended genes and the new recommendations. Some of the genes listed were included primarily for carrier status where an affected individual does not display symptoms of the disease but have a high likelihood of passing a severe disease to offspring. The reasoning behind including these genes is very similar but the genes themselves have different effects on the body. Each gene listed can cause this disease if mutated by itself. One example of a disease that has a multitude of genes associated with it is Arrhythmogenic right ventricular cardiomyopathy (ARVC). This disease is an example of a severe heart disease and is not unlike the other heart conditions that have been known to cause sudden death.

Arrhythmogenic right ventricular cardiomyopathy is a disease where fatty tissues replace cardiomyocytes in the right or both ventricles of the heart and symptoms include cardiac irregularities, syncope and sudden death. Multiple genes are correlated to this disease leading to different types or variants that each gene is causative of. The five known genes responsible for ARVC are TMEM43, DSP, PKP2, DSG2, DSC2. The TMEM43 gene is responsible for ARVC type 5 and is a response element for an adipogenic transcription factor. A loss of function in this gene is suggestive of how fibrofatty replacement occurs within the heart.[48] DSP causes ARVC type 8 and has been found connected to other diseases like Kertoasis Palmoplantaris Striata II, Wolly Hair, Lethal Acantholytic Epidermolysis Bullosa

[48] Bengtsson, Luiza, and Henning Otto. "LUMA Interacts with Emerin and Influences Its Distribution at the Inner Nuclear Membrane." *Journal of Cell Science* 4, no. 124 (2007): 538–46. doi:10.1242/jcs.019281.

and Keratoderma. The DSP protein is a part of the desmosome intermediate filament complex which connects cells together and is a part of the insoluble core. Homozygous and heterozygous mutations in this gene are known to cause the diseases listed up above. Some phenotypes of this disease involve rapid degeneration to heart failure at the age of four. Many different mutations, both homozygous and heterozygous, have been identified as causative for severe diseases in the DSP gene and making this an important gene to report to patients.[49] PKP2 is also associated with the desmosome complex as well as participates in linking cadherins to intermediate filaments. Mutations in this gene cause ARVC type 9 and patients exhibiting PKP2 mutations show arrhythmia earlier non-PKP2 mutations. PKP2 mutation inheritance is autosomal dominant so one mutated allele has the power to create the disease in an individual.[50] The fourth gene is DSG2, which is associated with ARVC type 10 and is also a part of the desmosome. ARVC caused from this gene is autosomal dominant and dysfunctional DSG2 proteins have been found from many different types of mutations within the gene. [51] The last gene is DSC2 which is another gene important to the desmosome complex and causes ARCV type 11. Studies on zebrafish embryos of DSC2 knockouts showed DSC2 was crucial in developing normal cardiac tissues and lead to myocardial contractility defects. Similar to PKP2, this gene has been implicated in other hereditary diseases like

[49] O'Neill, Marla J. F. "DESMOPLAKIN; DSP." *OMIM*, 2015. http://www.omim.org/entry/125647?search=DSP&highlight=dsp.
[50] O'Neill, Marla J. F., and Patti M. Sherman. "PLAKOPHILIN 2; PKP2." *OMIM*, 2008. http://www.omim.org/entry/602861?search=PKP2&highlight=pkp2.
[51] McKusick, Victor A., and Paul J. Converse. "DESMOGLEIN 2; DSG2." *OMIM*, 2011. http://www.omim.org/entry/125671?search=DSG2&highlight=dsg2.

palmoplantar keratoderma and woolly hair.[52] All of these genes are autosomal dominant and are causative of a severe disease that causes sudden death in individuals. The severity and ease of obtaining the disease are indicative of why these five genes should be included in secondary findings reports.

MYH11, MYLK and ACTA2 are another set of genes that are associated with heart disease. These genes are associated with familial thoracic aortic aneurysm with dissection (TAAD) 4, 7, and 6 respectively. This disease is associated with the dilation of the aorta and has been known to suddenly rupture making this a serious cardiac condition. Symptoms can show up anywhere from childhood to late adulthood. MYH11 encodes for the smooth muscle myosin heavy chain and is found all throughout the body. The MYH11 gene is connected to the CBFB gene which encodes for the CBF-Beta protein. Mutations in this region of MYH11 and the CBFB gene are associated with acute myeloid leukemia, making mutations in the MYH11 significant[53] and enough to report as a secondary finding. Another factor in the significance of this disease is that it is heterogeneous, meaning one affected allele in the individual is enough to cause the disease. ACTA2 encodes for an alpha actin found in skeletal muscle and constitute a major part of the contractile apparatus[54]. Both MYH11 and ACTA2 are a part of the smooth muscle cell contractile unit affecting the aorta,

[52] McKusick, Victor A., and Marla J. F. O'Neill. "DESMOCOLLIN 2; DSC2." *OMIM*, 2012. http://www.omim.org/entry/125645?search=DSC2&highlight=dsc2.

[53] Liu, P. Paul, Lucio H. Castilla, Lisa Garrett, Neeraj Adya, Donald Orlic, Amalia Dutra, Stacie Anderson, Jennie Owens, Michael Eckhaus, and David Bodine. "The Fusion Gene Cbfb-MYH11 Blocks Myeloid Differentiation and Predisposes Mice to Acute Myelomonocytic Leukaemia." *Nature Genetics* 23, no. 2 (October 1, 1999): 144–46. doi:10.1038/13776.

[54] "ACTA2 Actin, Alpha 2, Smooth Muscle, Aorta." *NCBI*. Accessed March 11, 2016. http://www.ncbi.nlm.nih.gov/gtr/genes/59/.

however one study suggested that mutations in either or both of these genes accounted for only around 20% of all familial TAAD diagnoses. Guo et al. (2007) suggested that other unknown mutations are significant factors in whether an individual develops familial TAAD.[55] The MYLK gene encodes for myosin light chain kinase which is a key enzyme in muscle contraction. This gene has also been associated with cases of familial TAAD where Wang et al. (2010) identified two heterozygous variants that seemed to be causative.[56] The MYLK and MYH11 genes have been known to be heterozygous and correlated with familial thoracic aortic aneurysms meaning one copy of these mutated genes are causative. The disease can cause sudden death making these mutations possible significant to a patient and should be reported. While studies suggest that they account for a low amount familial TAAD cases, these are still significant enough to inform patients about their chances of developing a cardiovascular disease. The ignorance of other causes of this disease makes reporting these genes only likely significant, however beforehand knowledge of susceptibility to familial TAAD is reason enough to be included in a report. The ACTA2 gene is connected with the MYH11 encoded protein so it should be reported along with the other two.

One of the most discussed sets of genes that affect a large number of people around the world are the BRCA1 and BRCA2 genes. These genes are known as tumor suppressors and play roles in transcription, DNA repair and recombination. The BRCA1 gene is a part of

[55] Guo, Dong-Chuan, Hariyadarshi Pannu, Van Tran-Fadulu, Christina L Papke, Robert K Yu, Nili Avidan, Scott Bourgeois, et al. "Mutations in Smooth Muscle α-Actin (ACTA2) Lead to Thoracic Aortic Aneurysms and Dissections." *Nature Genetics* 39, no. 12 (December 11, 2007): 1488–93. doi:10.1038/ng.2007.6.

[56] Wang, Li, Dong-Chuan Guo, Jiumei Cao, Limin Gong, Kristine E Kamm, Ellen Regalado, Li Li, et al. "Mutations in Myosin Light Chain Kinase Cause Familial Aortic Dissections." *The American Journal of Human Genetics* 87 (2010): 701–7. doi:10.1016/j.ajhg.2010.10.006.

a genome surveillance complex and is responsible for around 40% of breast cancers and a larger percentage of ovarian cancers. The TP53 tumor suppressor gene works with the BRCA1 gene and mutations in both of these are found in 70%-80% of BRCA1 mutated breast cancers.[57] Having non-functional BRCA1 gene has also been implicated in pancreatic cancers. The BRCA2 gene is similar and plays a role in completion of cell division and DNA repair. With breast cancer, a mutated BRCA2 gene also shows connections with early onset prostate cancer in males.[58] Both genes show autosomal dominant inheritance and both have high rates of cancer making them important to report to patients. These are only a few of the diseases on the ACMG's list of secondary findings but they are inclusive of the general attitude that went into choosing which diseases were included.

Proposed additions to the ACMG list

Rationale. This small set of the listed genes is an example of the reasoning behind why the ACMG included these genes in their list on secondary findings. The list of experts that made up the list focused on the severity of the disease, when the symptoms present themselves and the degree of deleteriousness witnessed in known mutations. All the genes recommended by the ACMG are included in this list due to these factors. New recommendations were chosen with these criteria in mind as well as being monogenic. Included in this list are carrier status genes where the patient or the family has a mutated

[57] McKusick, Victor A., and Ada Hamosh. "BREAST CANCER 1 GENE; BRCA1." *OMIM*, 2016. http://www.omim.org/entry/113705?search=BRCA1&highlight=brca1.
[58] McKusick, Victor A., and Ada Hamosh. "BRCA2 GENE; BRCA2." *OMIM*, 2016. http://www.omim.org/entry/600185?search=BRCA2&highlight=brca2.

chromosome that has a high likelihood to passing down and causing disease to future offspring. The reason for including carrier genes is for providing the patient with useful information in future planning and save offspring from debilitating diseases. If a patient knows that they have a 50% chance of passing a Mucopolysaccharidosis type II to a future male child, this information would hopefully influence their decision making. Inheritance patterns for these carrier status genes tend to be X-linked or autosomal dominant. Many of the new genes and diseases that I recommend are X-linked monogenic disorders. In studying monogenic disorders, a large proportion of them that were not included on the ACMG list were X chromosome linked. Because the mother is generally the one who passes down a problematic gene due to females having two X chromosomes, X-linked disorders oftentimes fit into the category of carrier status genes.

MPS II (IDS) One of these carrier status genes is the is the IDS gene which, when mutated, causes the disease Mucopolysaccharidosis type II (also known as Hunter syndrome) which has been mentioned multiple times. Mucopolysaccharidosis type II is a lysomal storage disorder because inactivation of Iduronate 2-sulfatase. This is the enzyme which the IDS gene encodes for and leads to a buildup of glycosaminoglycans. These molecules disrupt regular functions of a cell and lead to the distinct phenotype of Hunter Syndrome.[59] The inheritance of this disease is X-linked recessive and mostly affects males who inherit only one X chromosome from their mother. The life expectancy of an individual is only around

[59] Bocchini, Carol A. "IDURONATE 2-SULFATASE; IDS." *OMIM*, 2010. http://www.omim.org/entry/300823?search=IDS&highlight=ids.

10-20 years because of the progressive degeneration of function.[60] This is a severe disease and represents only one monogenic disorder that comes from mutations in the X chromosome.

Thalassemia and Fragile-X (ATRX, FMR1) The ATRX gene is also found on the X chromosome and is associated with the disease combination alpha thalassemia/mental retardation syndrome. The ATRX gene encodes for a protein within the helicase family and is believed to be associated with the regulation of gene expression and sex differentiation. Male patients show varying degrees of undeveloped sexual organs. Mutations that happen to truncate the gene and eliminate the C-terminal region tend to come with the genital defects.[61] Many different mutations have been implicated in this disease[62] which makes this gene important to report to patients. While this disease is more likely to affect males, it has been known to affect females because of X-chromosome inactivation where one X chromosome copy is inactivated in order to prevent overexpression. The importance of reporting this gene lies with the carrier status of a mutant allele. Fragile X syndrome is a very similar disease that is also X-linked caused by mutations in the FMR1 gene. This syndrome's phenotype is moderate to high mental disabilities and patients tend to exhibit autistic behavior. FMR1 encodes for a protein thought to be involved in translation and is involved

[60] "Mucopolysaccharidosis Type II." US National Library of Medicine, National Institutes of Health, Department of Health & Human Services, 2016. https://ghr.nlm.nih.gov/condition/mucopolysaccharidosis-type-ii.

[61] Picketts, D J, D R Higgs, S Bachoo, D J Blake, O W Quarrell, and R J Gibbons. "ATRX Encodes a Novel Member of the SNF2 Family of Proteins: Mutations Point to a Common Mechanism Underlying the ATR-X Syndrome." *Human Molecular Genetics* 5, no. 12 (1996): 1899–1907. doi:6d0165 [pii].

[62] McKusick, Victor A., and Ada Hamosh. "ATR-X GENE; ATRX." *OMIM*, 2016. http://www.omim.org/entry/300032?search=ATRX&highlight=atrx.

in neuron and hippocampus development[63]. Like alpha thalassemia/mental retardation syndrome, this disease primarily affects males because of their singular copy of the X chromosome but can also affect females. Also like the afore mentioned disease, it is important to inform patients if they are carrying a mutated copy of the gene. Reporting carrier status to a patient or their family may be an important piece of information if they are considering having a child. Mental handicaps are a serious issue for some people and the burden may be more than people are willing to risk. Preimplantation genetic diagnosis is already an established practice for in-vitro fertilization where genetic tests are run on the embryo which test for specific diseases or conditions.[64] PGD is representative of the acceptance for scanning a fetus for conditions that would make life hard on the parents. This issue is seen in a few more new recommendations found on the list.

Duchene and Becker Muscular Dystrophy (DMD) The DMD gene is associated with two related diseases, Duchenne Muscular Dystrophy and Becker Muscular Dystrophy. Both diseases involve progress muscle loss and resulting weakness with Becker muscular dystrophy being the milder form. DMD encodes for dystrophin which is believed to help actin maintain its bonds while under bending stress. Many different types of mutations have

[63] Abitbol, Marc, Christian Menini, Anne-Lise Delezoide, Thomas Rhyner, Michel Vekemans, and Jacques Mallet. "Nucleus Basalis Magnocellularis and Hippocampus Are the Major Sites of FMR-1 Expression in the Human Fetal Brain." *Nature Genetics* 4, no. 2 (June 1993): 147–53. doi:10.1038/ng0693-147.

[64] Hershberger, Patricia E, Catherine Schoenfeld, and Ilan Tur-Kaspa. "Unraveling Preimplantation Genetic Diagnosis for High-Risk Couples: Implications for Nurses at the Front Line of Care." *Nursing for Women's Health* 15, no. 1 (2011): 36–45. doi:10.1111/j.1751-486X.2011.01609.x.

been recorded to cause both of these diseases[65] which may not appear until later in life. While this disease may not necessarily be life threatening (Duchenne muscular dystrophy has been known to shorten life expectancy), it has a major effect on the life of individuals. The loss of muscle over time affects patients negatively and it is best to inform patients of what they may experience in the future.

IP and Norrie Disease (IKBKG, NDP) Incontinentia Pigmenti (IP) is an X-linked dominant disease that primarily affects the skin where the skin is inconsistently pigmented with sporadic blisters and rashes. It has also been known to influence abnormally developed eyes and intellectual disability. Symptoms sometimes affect newborns but progressive elements occur later in life and primarily affects females[66]. Males with the mutation do not tend to make it past the embryonic stage of development. The gene responsible is IKBKG which encodes for a NEMO-like kinase which specifically phosphorylate serine and threonine that are followed by a proline. Many mutations seem to be deletions within the gene which cause Incontinentia Pigmenti but this gene is also associated with Ectodermal dysplasia, hypohidrotic, with immune deficiency which proves to be a serious issue in affected patients. Affected males show high rates of morbidity and mortality due to recurrent

[65] Hartz, Patricia A. "DYSTROPHIN; DMD," 2016. http://www.omim.org/entry/300377?search=DMD&highlight=dmd.

[66] "Incontinentia Pigmenti." *National Institutes of Health*. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services, 2008. https://ghr.nlm.nih.gov/condition/incontinentia-pigmenti.

infections[67]. While mutations in this gene are non-lethal for affected females, affected males have a high mortality rate and patients carrying the mutation should be informed that they are carriers. A similar disease that affects mental capabilities and blindness is Norrie disease. The gene associated with Norrie disease is NDP which encodes for norrin which is a cysteine rich protein associated with the cysteine knot growth factor family.[68] Norrie Disease is X-linked recessive and causes blindness in affected males at birth as well as can lead to progressive hearing loss and mental handicaps. Like IP, mutations in the NDP gene should be reported to patients who carry the mutation which in this case are females. Patients will have more information regarding whether they should have offspring that may be affected with these diseases so it is important to inform them of mutations in these genes.

Ornithine Transcarbamylase Deficiency (OTC) Ornithine transcarbamylase deficiency is caused by mutations in the OTC gene which encodes for ornithine carbamoyltransferase and is involved in the urea cycle of mammals. Deficiency in this protein leads to increased levels of ammonia in the body which negatively affects the nervous system. Complications from this gene lead to an umbrella of problems for the patient from intellectual disability to lethargy to progressive liver damage[69]. Symptoms usually show

[67] Zonana, Jonathan, Melissa E. Elder, Lynda C. Schneider, Seth J. Orlow, Celia Moss, Mahin Golabi, Stuart K. Shapira, et al. "A Novel X-Linked Disorder of Immune Deficiency and Hypohidrotic Ectodermal Dysplasia Is Allelic to Incontinentia Pigmenti and Due to Mutations in IKK-Gamma (NEMO)." *The American Journal of Human Genetics*. Vol. 67, 2000. doi:10.1086/316914.

[68] Meindl, Alfons, Wolfgang Berger, Thomas Meitinger, Dorien van de Pol, Helene Achatz, Christa Dörner, Martina Haasemann, et al. "Norrie Disease Is Caused by Mutations in an Extracellular Protein Resembling C–terminal Globular Domain of Mucins." *Nature Genetics* 2, no. 2 (October 1992): 139–43. doi:10.1038/ng1092-139.

[69] "Ornithine Transcarbamylase Deficiency." *Genetics Home Reference*. US National Library of Medicine, National Institutes of Health, Department of Health & Human Services, 2006. https://ghr.nlm.nih.gov/condition/ornithine-transcarbamylase-deficiency.

up at birth but are sometimes not caught until later in life. Males are usually the ones most affected by this disease but females can also be affected, often through X inactivation[70].

Spinal and Bulbar Muscular Atrophy (AR) Similar to Ornithine transcarbamylase deficiency, Spinal and bulbar muscular atrophy primarily affects males. Symptoms of this disease include muscle weakness and wasting which occurs in adulthood. The AR gene is responsible for Spinal and bulbar muscular atrophy and encodes for the androgen receptor protein involved with androgen response elements. Mutations in AR have also been associated with Androgen insensitivity syndrome and susceptibility to prostate cancer.

Wiskott-Aldrich syndrome (WAS) The final X-linked disorder is Wiskott-Aldrich syndrome that is characterized by an abnormal immune system and problems forming blood clots. WAS is the gene associated with this disease and is involved in Cdc42 signaling. Patients with Wiskott-Aldrich have a reduced number of platelets in their body and is caught early on in life because of ease of bleeding and bruising. They also have an increased chance of developing autoimmune disorders. The only known way to treat this disease is a bone marrow transplant[71].All of these monogenic disorders have severe consequences for patients, usually males, that inherit mutated genes from their parents. Patients carrying these diseases should be informed that they have the capacity to pass debilitating or deadly problems on to their offspring.

[70] Kniffen, Cassandra L., and Victor A. McKusick. "ORNITHINE CARBAMOYLTRANSFERASE; OTC," 2006. http://www.omim.org/entry/300461?search=OTC&highlight=otc.

[71] "Wiskott-Aldrich Syndrome in Children." *Boston Children's Hospital*. Accessed March 29, 2016. http://www.childrenshospital.org/conditions-and-treatments/conditions/w/wiskott-aldrich-syndrome.

Gerstmann-Straussler disease (PRNP) Non X-linked recommendations that I made within this list include Gerstmann-Straussler Disease, Cystic Fibrosis, Huntington's Disease and acute myeloid Leukemia brought about by KRAS. Gerstmann-Straussler disease is a prion disease that affects the nervous system and includes ataxia, cognitive dysfunction and spasticity. Symptoms develop around the ages of 35 to 50 and patients usually live an average of 5 years after diagnosis[72]. The PRNP gene is associated with this disease as well as Creutzfeldt-Jakob disease and a Huntington-like disease. PRNP encodes for a glycoprotein that is attached to the plasma membrane. The functional form of this protein has a large alpha-helical structure and the pathogenic isoform in beta-pleated sheets[73].

Cystic Fibrosis (CFTR) Cystic Fibrosis is associated primarily with the CFTR gene which encodes for an ATP-binding cassette and is involved in chloride ion channels. Characteristics of this disease include buildup of mucus in the lungs and digestive problems. Symptoms are present from birth and modern medical advancements have allowed patients with Cystic Fibrosis to live into adulthood[74]. The severity of the disease is likely influenced by the presence of mutations in other genes.

Huntington's Disease (HTT) Another severe disease that develops well into adulthood is Huntington's disease which is brought about by mutations in the HTT gene. HTT encodes

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[72] Mastrianni, James A. *Genetic Prion Diseases*. *GeneReviews(®)*. University of Washington, Seattle, 1993. http://www.ncbi.nlm.nih.gov/pubmed/20301407.

[73] Vanik, D. L., and W. K. Surewicz. "Disease-Associated F198S Mutation Increases the Propensity of the Recombinant Prion Protein for Conformational Conversion to Scrapie-like Form." *Journal of Biological Chemistry* 277, no. 50 (December 13, 2002): 49065–70. doi:10.1074/jbc.M207511200.

[74] "Cystic Fibrosis." US National Library of Medicine, National Institutes of Health, Department of Health & Human Services, 2016. https://ghr.nlm.nih.gov/condition/cystic-fibrosis.

for huntingtin which is a nuclear protein that regulates transcription factors. There is a CAG repeat that is often found within Huntington's patients and the disease seems to have a gain-of-function aspect to it where the encoded mRNA or resulting protein have a new property or express inappropriately.[75] Symptoms of this disease include loss of motor function, emotional capabilities and intellectual abilities; and eventually leads to death. This disease is autosomal dominant so it is likely that a patient whose parent is a carrier of the disease has a 50% chance of obtaining the mutated carrier gene.[76]

Acute Myeloid Leukemia (KRAS) The last gene recommended was KRAS in association with acute myeloid Leukemia. Mutations in KRAS are associated with many different types of cancers so this gene increases cancer susceptibility. This type of leukemia is adult onset and causes bone marrow to produce abnormal myoblasts. KRAS encodes for a protein involved in tissue signaling and play a role in proliferation, differentiation and senescence. Mutated forms of KRAS are prolific oncogenes that have been associated bladder, breast, gastric and lung cancers as well as Noonan syndrome and acute myeloid leukemia. The inheritance pattern for acute myeloid leukemia is known to be autosomal dominant while for other cancers the inheritance in not known. The severity of all of these diseases and the fact that they are adult-onset or can be are reason to include mutations of these genes in secondary findings reports.

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[75] MacDonald, Marcy E., Christine M. Ambrose, Mabel P. Duyao, Richard H. Myers, Carol Lin, Lakshmi Srinidhi, Glenn Barnes, et al. "A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes." *Cell* 72, no. 6 (1993): 971–83. doi:10.1016/0092-8674(93)90585-E.

[76] "Huntington Disease." *U.S National Library of Medicine*. Accessed March 29, 2016. http://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0024706/.

Conclusion

The goal of this thesis is to convince institutions, and especially the Rare Genomics Institute, to implement policies of providing patients with reports on secondary findings. In having the ability to easily provide more information to patients but neglecting to do so, institutions and medical professionals are doing a disservice to their patients. We are morally obligated to act in the greatest beneficent capacity that we can as medical professionals. Giving patients as much knowledge about their own body as possible is acting in the most beneficent way because patients have the autonomy to do what they will with that information. Secondary findings should be encouraged and offered to all patients who undergo genetic testing. Information is power and patients gain more power over their situation when they know what they can experience. Federal laws like the Genetic Information Nondiscrimination Act is at the lowest limit of what is acceptable protection for individuals. Further protections for patients are needed but the information that is gathered from secondary findings reports are invaluable just the same.

The general consensus amongst the literature is that secondary findings should be reported and part of this report uses RG as an example of how to approach the issue with patients. Because RG does not currently deal with secondary findings it may be a rough transition to beginning to offer them. Cases that are currently being worked on would likely receive secondary findings later than their analysis report but even these liminal state cases

should be offered secondary findings. Cases that have already been closed should also be offered reanalysis of their genetic data because oftentimes the institution still has the data and the contact information of the patient. The speed of transition could affect whether some patients are offered the secondary findings report in a timely manner. Filters in databases or during sequencing will speed up the process tremendously and there are already some filters for the ACMG list like in Omicia Opal.

More problematic genes can be expected to show up on this list of secondary findings from the work that's being done on monogenic disorders around the world. Reported genes may expand to well understood polygenic disorders within the next few decades and the role of unknown elements like introns could be determined and included. I hope to continue research that contributes to the understanding of hereditary diseases. There is much more work to be done before finalizing the list of what should be given to patients. The factors of time, cost and availability of genetic sequencing contribute to the growth and use of genetic data in diagnosing diseases in contemporary medical practices. The future of genetic analysis is looking promising and institutions like Rare Genomics need to be using this technology to their best ability and providing secondary findings to patients to fulfill their express purpose of helping those in need.

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